The University of Chicago

A PHYSIOLOGICAL AND CHEMICAL STUDY OF AFTER-RIPENING

A DISSERTATION

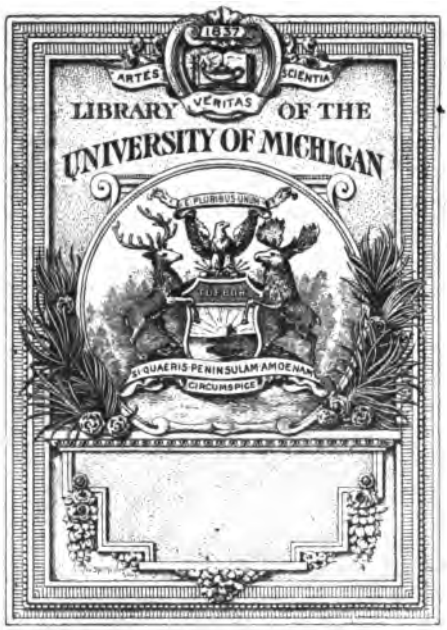
SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY

SOPHIA ECKERSON

Reprinted from
THE BOTANICAL GAZETTE, Vol. LV, No. 4
Chicago, 1913


1837
ARTES
VERITAS
SCIENTIA
LIBRARY OF THE
UNIVERSITY OF MICHIGAN
E PLURIBUS UNUM
SI QUAERIS PENINSULAM AMOENAM
CIRCUMSPICE

The University of Chicago

A PHYSIOLOGICAL AND CHEMICAL STUDY OF AFTER-RIPENING

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY
SOPHIA ECKERSON

Reprinted from
THE BOTANICAL GAZETTE, Vol. LV, No. 4
Chicago, 1913

A PHYSIOLOGICAL AND CHEMICAL STUDY OF AFTER-RIPENING

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 170

SOPHIA ECKERSON

Many seeds and spores require a long time for germination. The term "after-ripening" has come into rather general use to designate the changes in the seed during this period. It is often loosely used to include disintegration of the seed coats as well as protoplasmic or metabolic changes in the embryo. It seems better to limit its use, as has been done in this laboratory, to those cases where the delay is due to characters of the embryo. In the majority of seeds thus far investigated, the delayed germination is due to the exclusion of water or of oxygen by the seed coats. A few seeds have been studied, however, which do not grow when all coats have been removed and the embryo put in good germinating conditions. Some change within the embryo is necessary before germination, that is, lengthening of the hypocotyl, can take place. This process is what we mean by "after-ripening."

NOBBE and HANLEIN (**45**), WIESNER (**52**), JOST (**29**), and others assume, in cases where water enters the seed coat, that growth after a long period is due to some change going on within the embryo during the seemingly dormant period. This has been determined definitely for only three or four species.

LAKON (**35**) finds that the delayed (1–2 years) germination of *Pinus silvestris*, *P. Strobus*, and *P. Cembra* is not due to coat characters. With the coats broken or removed, the time required for germination was not shortened. Seeds of *Fraxinus excelsior* (**36**) sown in the spring do not germinate until the following spring. In the mature seed the embryo occupies about half the space within the endosperm; the rest is occupied by a mucilaginous substance. During the year that the seed lies in the ground, the embryo grows in length and fills the seed coat. Since the embryo is fully mature at maturity of the seed, but a period of growth is

necessary before germination, LAKON calls this "Vorkeimung" instead of "Nachreife."

Many methods have been used by different workers in the attempt to shorten the resting period of buds and bulbs. The first of these is JOHANNSEN'S (30) treatment with ether. He found that growth could be hastened at the beginning of and near the end of the resting period, but not in the middle period. MOLISCH (40) immersed shoots in water at 35° C., and the buds opened earlier than those on untreated shoots. IRAKLIONOW (23) finds that the warm bath increases respiration only in the first days, then the respiration curve falls to its original height. MÜLLER-THURGAU and SCHNEIDER-ORELLI (42, 43) used the warm bath method to hasten the germination of potato tubers, and lily of the valley bulbs. They find the sugar content increased by the warm bath, but this is immediately used by the increased respiration. Unless the bulbs are kept at a high temperature, there is no lasting effect. Zero temperature increases the sugar content and the respiration; injury produces a slight increase in sugar content. They do not believe that hastened growth in these cases is due to increased sugar content, but rather that the high temperature has some effect on the protoplasm. CHRISTENSEN (3) made chemical analyses of resting and growing bulbs, but found no appreciable differences. He concluded that the slow growth of resting bulbs was not due to lack of soluble food materials.

WEBER (53) and JESENKO (27) found that injury to the buds hastens their development. Later, JESENKO (28) found that the shoots from these buds were abnormal. Shoots immersed in dilute solutions of alcohol, H_2SO_4, and other substances, develop normally and more rapidly than untreated shoots. LAKON (37) forced the development of winter buds by standing the cut ends of shoots in Knop's solution. MOLISCH (41) has recently found radium emanations effective.

All these various methods shorten somewhat the rest period of bulbs and winter buds. Little has been done to determine what is the limiting factor to growth in these cases and what internal change is produced by the external application.

CROCKER (4, 5) found that the long period required for germina-

tion of seeds of hawthorn is due in part to seed coat characters and in part to characters of the embryo. With testas removed, in light at room temperature, the cotyledons increase greatly in size and turn green, but only a small percentage (2–5 per cent) of the hypocotyls grow.

DAVIS and ROSE (**6**), working in this laboratory, studied further the germination of seeds of *Crataegus mollis*. They find that under ordinary conditions seeds with carpels intact require one year or more for germination. Embryos with carpels removed, but with testas intact, germinated after 90–96 days at 5–6° C.; 74 per cent germinated after 75 days in the cold when removed to the warm greenhouse. With both carpels and testas removed, after 28 days at 6° C. 78 per cent germinated in 5 days in the greenhouse. Thus with all coats removed there is still a delayed germination due to characters of the embryo itself, a period of "after-ripening" necessary before elongation of the hypocotyl can take place. It has been my purpose to study the changes within the embryo during this period.

Investigation

I have made a preliminary microchemical study of the chemical changes during after-ripening. These results form the basis for a quantitative study. This paper gives the results of the microchemical study, together with quantitative determinations of the substances in the embryo at different periods during after-ripening.

DAVIS and ROSE (**6**) found the best conditions for after-ripening to be a temperature of 5–6° C. This inhibits growth of the cotyledons and is favorable for the metabolic changes within the hypocotyl. I studied the after-ripening of several species of *Crataegus*.

Seeds with testas intact were soaked 16 hours at 5° C., were washed thoroughly by shaking in a bottle of distilled water (to prevent mold), and put in dishes on moist cotton in the ice chest at 5–6° C. Microchemical tests were made once a week during the after-ripening period. Sections were preserved in glycerin for comparison.

MICROCHEMICAL METHOD

In order to detect the metabolic substances of the cell as nearly unchanged as possible, observations must be made on the living tissue. Sections were made on a freezing microtome or free-hand, and *intra-vitam* stains (**8**) were used. Of the different methods and stains used, the following were found to be the most valuable.

Fats.—Soudan III or Scharlach R, dissolved in 50 per cent alcohol. All fats are soluble in these stains.

Lecithin.—The fats were first dissolved out with acetone, in which lecithin is not soluble. It was then stained with Soudan III, or blackened with fumes of osmic acid.

Starch.—The sections were first heated in water on the slide, and then a drop of iodine solution added.

Sugar.—For the reducing sugars Fehling's solution was used as follows. The sections were heated in the copper sulphate solution, on the slide. The other part of Fehling's solution, Rochelle salt and sodium hydroxide, was then added. Copper oxide is deposited in the cells containing sugar. Still better is the osazone test as modified by MANGHAM (**38**). Dissolve phenylhydrazine hydrochloride and sodium acetate in 10 times their weight of glycerin; warm to dissolve; filter once or twice. To use, put a drop of each on the slide, mix, put section of tissue in same. Place slide in warm oven 5–30 minutes, and then cool. Osazone crystals will be formed in the cells which contained sugar.

Acidity or alkalinity.—Neutral red, sulphate of Nile blue, Dahlia violet, and methyl orange. A 1/5000 solution of neutral red is sensitive to N/11,000 NaOH; a 1/5000 solution of sulphate of Nile blue is sensitive to N/5100 NaOH; the others are less sensitive (**9**).

Catalase.—A drop of H_2O_2 was put on the section on the slide and evolution of oxygen noted.

Oxidase.—A few drops of freshly made solution of guaiaconic acid, on the slide.

Peroxidase.—A drop of H_2O_2 added to a few drops of guaiaconic acid, put on the sections on the slide.

TABLE I

CONDITION OF THE EMBRYO OF CRATAEGUS GLORIOSA

Glucoside amygdalin	Condition of seeds	Fatty oil	Lecithin	Protein	Sugar	Starch	Catalase	Peroxidase	Oxidase	Acidity
HCN.....	Air-dry	Abundant (25 to 30 per cent)	x	x	o	o	x	Hyp. little Cot. x	o	Hyp. basic Cot. acid
	After-ripened	Slight decrease	x	x	Trace	o	Slight increase	Great increase	x	Great increase
	At germination	Decrease	x	x	x	x	x	x	x	x

The value of neutral red as an indicator has been questioned by many. If the change in color of a dye is due to H ion content, it can safely be used as an indicator. FRIEDENTHAL (13) gives a very valuable series of indicators with the H or OH ion concentration which will produce a change in color. In the presence of substances which form combinations with the dye, the change in color would be no indication of H or OH ions. In all my work, however, the acidity or alkalinity as shown by neutral red was confirmed by titrations with NaOH or HCl.

Microchemical tests should be followed always by quantitative determinations at the critical points. When so used the method is reliable and is a great saving of time and material.

RESULT OF MICHROCHEMICAL TESTS

The condition of the embryo at the beginning and end of the after-ripening period, and at germination just after the hypocotyl has pushed out through the testa, is shown in the accompanying table (I). In all the tables, *x* indicates presence in quantity, and o indicates absence.

There is a very gradual, though constant, increase in the acidity and in the enzymes during the whole period. After 80–90 days at 5° C.,

when the acidity has almost reached its maximum, the fats begin to break up and sugar appears. Oxidase first appears at this time. Hydrocyanic acid appears after 75 days, increases up to germination, then decreases (**19, 49**).

WATER-HOLDING POWER

Since the hypocotyls of the air-dry seeds are basic, while the cotyledons are acid, it was thought that they might have less ability to take up water than the cotyledons. To test this, seeds were taken at different stages of after-ripening, the coats removed, and the naked embryos soaked in water for 5 hours. These were then dried carefully with filter paper and, after the hypocotyls were separated from the cotyledons, were put in separate weighing bottles. These were weighed, then heated in an oven 10 minutes at 100° C., removed from the oven, and dried at 50° C. Many determinations were made. The accompanying table (II) gives a typical series.

TABLE II

WATER-HOLDING POWER OF EMBRYO OF CRATAEGUS GLORIOSA

	Hypocotyls			Cotyledons		
	Wet weight grams	Dry weight grams	Percentage water	Wet weight grams	Dry weight grams	Percentage water
Coats removed and soaked 5 hrs.	0.0157	0.0121	23	0.2690	0.1542	42
90 days at 5° C.	0.0189	0.0116	38	0.2700	0.1744	35
Germinated (hyp. 3 mm.)	0.0289	0.0115	60	0.2094	0.1260	39

ACIDITY

Determinations of acidity were made at the same periods of after-ripening as the water determinations (table III). The embryos were washed in distilled water which had been boiled to expel CO_2.

It will be seen that the metabolism of the fats does not begin until the acidity has reached a certain amount, the water content has increased greatly, and the enzymes are set free. The hypocotyl does not elongate until that time. It has long been known that

considerable free fatty acid is formed during the germination of oily seeds (GREEN **17**, SCHMIDT **50**). MILLER (**39**) finds, in the germination of *Helianthus annuus*, that the quantity of free acid in the hypocotyl increases rapidly at the beginning of germination.

IVANOW (**24, 25, 26**) studied the metabolism of fats in ripening and in germinating seeds. He finds that the rapidity of oil transformation in germinating seeds depends on the fatty acid components of that particular oil. The oil of flax and of hemp contains

TABLE III

ACIDITY OF EMBRYO OF CRATAEGUS GLORIOSA

	Hypocotyls			Cotyledons			
	Grams	N/20 KOH cc.	N/20 KOH cc. per gram		Grams	N/20 KOH cc.	N/20 KOH cc. per gram
Air-dry	0.0267	Slightly basic	0.00	Air-dry	0.5374	0.20	0.37
30 days	.....	.....	0.10	30 days	.....	....	0.40
60 days	.....	.....	0.30	.....	.....	....	....
90 days at 5° C. (hyp. 2 mm.)	0.0444	0.04	0.90	Cots. white	0.8162	0.35	0.42
Germinated (hyp. 2.5 cm.)	0.360	0.45	1.24	Cots. yellow	0.1914	0.60	3.13
Germinated (hyp. 3.5–5 cm.)	0.5967	0.65	1.09	Cots. green (in light 1 week)	0.3907	1.20	3.07
Germinated (hyp. 3–5 cm.)	0.3810	0.60	1.57	.....	0.7099	1.90	2.67

the less saturated acids—linolenic and linoleic—and is transformed much more quickly than that of rape, which contains oleic acid. The less saturated acids disappear rapidly (forming carbohydrates) from the seedlings and cause the very low acid number of flax and hemp. Unsaturated acids of the oleic acid type are more stable and inactive; therefore rape shows a higher acid number than flax or hemp. The acids in the seeds of *Crataegus* were not identified; this will be done later.

DELEANO (**7**) studied the chemical changes during the germination of *Ricinus communis*. He finds that the acidity and the

catalase increase up to a maximum, which is reached on the eighth day of germination (the hypocotyl is then 2.5 cm. long). Then the fats begin to break up and within two or three days disappear. The fats are transformed into a soluble substance of the character of a plant mucilage. This is later transformed into sugar, cellulose, and other substances. DELEANO says that the acids activating hydrolysis are formed during germination; he detected acetic and lactic acids. He thinks that catalase is directly concerned with hydrolysis of the fats. This is doubtful, however, since catalase is so universally present. Peroxidase reached a maximum about the fourteenth day of germination (the hypocotyls were 8.5 cm.).

The chemical changes during the 90 days of after-ripening of *Crataegus* are the same as those of the first 8 days of germination of *Ricinus*. It is as though the chemical processes, telescoped in *Ricinus*, are drawn out in *Crataegus*.

Seeds of the crab apple (*Pyrus baccata*) after-ripen in 30 days at 5° C. At the beginning of their after-ripening period, hypocotyls of these embryos have an acidity and a water-holding power slightly greater than those of *Crataegus* after 60 days at 5° C. (hypocotyl 1.45 cc., N/20 KOH, moisture 48 per cent; cotyledons 0.368 cc., moisture 39 per cent). Peroxidase increases gradually from a very little in the air-dry seeds to a considerable amount at germination. As in *Crataegus*, oxidase does not appear in hypocotyls of *Pyrus baccata* until immediately before germination.

EFFECT OF ACIDS

FISCHER (11) finds that when seeds of water plants (*Alisma*, *Sagittaria*, and *Sparganium*) are treated with dilute solutions of acids, or the strong alkalies KOH and NaOH, the percentage of germination is increased. He conceives the H ions of the acids, and the OH ions of the alkalies as destroying the equilibrium of the cell and starting up the chemical processes.[1] Seeds treated with solutions of the fatty acids (formic, acetic, propionic, and butyric) did not germinate. He therefore considers these acids toxic; but he used too concentrated solutions. In dilute solutions the

[1] CROCKER has found that these seeds germinate readily if the coats are broken or removed. The protoplasm is not dormant.

fatty acids shorten the after-ripening period of both hawthorn and apple.

Mlle. PROMSY also used acetic acid with good results on seedlings of tomato and corn. She studied (**47, 48**) the effect of acids on the respiration of germinating seeds of tomato, corn, barley, *Dioscorea*, and *Elaeis guineensis*. She finds that organic acids (citric, malic, oxalic, tartaric, and acetic) increase the respiratory quotient and at the same time the intensity of respiration, measured by evolution of CO_2. Inorganic acids, on the other hand, do not modify the quotient, except in the single case of the fatty seeds of *Elaeis*. Seeds were soaked in a solution of the acid for 24–48 hours, then put in germinative conditions in sand, and the sand watered with the solution. Seedlings submitted to the action of organic acids grew more rapidly than the control, increased more in wet weight, and increased more in dry weight, if determined at the end of the germination period when the plants were green. Seeds treated with inorganic acids, HCl and H_2SO_4, germinate more quickly than the control. The wet weight of seedlings is increased, dry weight is the same as the control.

MARTIN FISCHER (**12**) found that acids greatly increase the absorption of water by colloids, while salts decrease the absorption. He studied the absorption of water by gelatin, fibrin, and frog muscle.

A certain degree of acidity seems to be necessary before germination of *Crataegus* seeds. The acidity of the hypocotyl develops very slowly and little water is absorbed in the early stages of after-ripening. It was thought that absence of free acids might be the limiting factor to growth. An attempt was made to supply this by soaking the seeds in acid before putting them in after-ripening conditions.

METHOD OF TREATMENT WITH ACIDS

Seeds of *Crataegus*, with carpels removed but testas intact, were soaked in the acid solution over night at 5° C., washed, and put on moist cotton in Petri dishes. At the end of 14 days they were washed carefully, the coats removed, and the embryos washed in distilled water. They were then tested for presence of free enzymes. In the accompanying table (IV) the results are given

in order of the depth of color of the reaction for peroxidase and oxidase.

TABLE IV

ENZYMES IN HYPOCOTYLS OF CRATAEGUS DUROBRIVENSIS

	Catalase	Peroxidase	Oxidase
Air-dry at 5° C. 14 days	Very little	Very pale	0
Control	Little	Light blue	0
N/1000 HCl	x	Light blue	0
N/100 acetic	x	Slightly darker	0
N/50 butyric	x	Slightly darker	?
N/70 butyric	x	Darker	Trace
N/3400 HCl	x	Darker	Trace
N/1000 acetic	x	Like after-ripened	Dark blue
After-ripened 90 days at 5° C	x	Dark blue	Dark blue
Germinated	x	Deep blue	Darker

In 14 days embryos treated with N/1000 acetic acid attain an enzyme reaction equal to that of the untreated embryo after 90 days' after-ripening. Both N/3400 HCl and N/70 butyric show a great increase in enzymes over the control.

The acidity was determined in several cases. The testas and embryos were always carefully washed in boiled distilled water to prevent any trace of external acid vitiating the results. The accompanying table (V) gives the results of one series.

TABLE V

ACIDITY IN EMBRYOS OF CRATAEGUS DUROBRIVENSIS

IN ACID OVER NIGHT	HYPOCOTYLS			COTYLEDONS		
	Grams	N/20 KOH cc.	N/20 KOH cc. per gram	Grams	N/20 KOH cc.	N/20 KOH cc. per gram
Control	.....	Slightly basic	....	.....	....	0.37
N/70 butyric 16 hours	0.0184	Neutral	0.00	0.3544	0.16	0.45
N/70 butyric 14 days at 5° C	0.009	0.01	1.11	0.3138	0.65	2.06
N/100 acetic 14 days at 5° C	0.0119	0.02	1.68	0.2420	0.40	1.65

After seeds have been in N/70 butyric acid 16 hours, the outer cells of the cortex of the hypocotyl give an acid reaction with Dahlia

violet. The inner cells are still basic. This shows that the acid had penetrated a little way. The inner cells develop acidity much more rapidly than in the control.

Of seeds treated as above with N/70 butyric acid, and after-ripened at 5° C. with testas on, 52 per cent germinated in 53 days; the others decayed. The testas were removed from a lot of seeds after soaking in N/70 butyric acid, and the naked embryos kept on moist cotton at 5° C.; these germinated in 16 days. As shown by the large number of seeds which were killed, N/70 butyric is too strong. More dilute solutions are being used now.

N/800 butyric has about the conductivity of N/1000 acetic and N/3400 HCl, which were found to be effective; therefore that is being tested as well as still more dilute solutions. The more dilute solutions are not toxic, instead they greatly increase the rate of the process of after-ripening; therefore, germination is hastened. The after-ripening period of seeds with testas intact was shortened from 80–90 days to 45–53 days; with testas removed, from 30 days to 16–18 days.

Summary

Condition of the embryo in dry storage.—Food is stored in the embryo in the form of fatty oil; there is also considerable lecithin; neither starch nor sugar is present. The reaction of the cotyledons is acid, but the hypocotyl is slightly basic. The water-absorbing power of the hypocotyl is less than 25 per cent of the wet weight.

There is a series of metabolic changes in the embryo during the period of after-ripening. The initial change seems to be an increased acidity. Correlated with this is an increased water-holding power, and an increase in the activity of catalase and peroxidase.

Near the end of the period of after-ripening there is a sudden increase in the acidity, and in the water content; here oxidase first appears. All of these increase until the hypocotyl is 3–5 cm. long. At this time the fats decrease and sugar appears. Hydrocyanic acid is present in the cotyledons.

The after-ripening period can be greatly shortened by treating the embryos with dilute acids, HCl, butyric, and acetic. The water-holding power, the acidity, and the amount of peroxidase increase much more rapidly, and oxidase appears much earlier, than in untreated embryos.

It is evident that there is a correlation between acidity of the hypocotyl of *Crataegus*, its water-absorbing power, production of enzymes, and germinating power. Whether the acidity is causal or merely correlative is not known. There is some evidence, however, that it is causal. GREEN (**17, 18**) has shown that it leads to the liberation of enzymes; and MARTIN FISCHER (**12**) that it increases the water-absorbing power of colloids. Other dormant seeds of the Rosaceae are now being studied with the hope of gaining further knowledge on this point.

Acknowledgments are due Dr. WILLIAM CROCKER, under whose direction the work was done.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. APPLEMAN, CHARLES O., Physiological behavior of enzyme and carbohydrate transformations in after-ripening of the potato tuber. BOT. GAZ. **52**:306–315. 1911.
2. BOS, H., Wirkung galvanischer Ströme auf Pflanzen in der Ruheperiode. Biol. Centralbl. **27**:673–681, 705–716. 1907.
3. CHRISTENSEN, P., Chemical researches of bulbs in the later phases of the resting period. Bull. Acad. Roy. Danemark (Copenhague). pp. 44. 1908.
4. CROCKER, WILLIAM, Rôle of seed coats in delayed germination. BOT. GAZ. **42**:265–291. 1906.
5. ———, Longevity of seeds. BOT. GAZ. **47**:69–72. 1909.
6. DAVIS, WILMER E., and ROSE, R. CATLIN, The effect of external conditions upon the after-ripening of the seeds of *Crataegus mollis*. BOT. GAZ. **54**: 49–62. 1912.
7. DELEANO, N. T., Recherches chemiques sur la germination. Centralbl. Bakt. **24**^{2}:130–147. 1909.
8. Enzyklopädie der mikroskopischen Technik. II. Vitale Färbung. pp. 589–602. 1910.
9. FAURÉ-FREMIET, E., Les mitochondries des Protozaires et cellules sexuelles. Arch. Anat. Mic. **11**:457–648. 1910.
10. FAWCET, H. S., Viability of weed seeds under different conditions of treatment and study of their dormant periods. Proc. Iowa Acad. 1908.
11. FISCHER, ALFRED, Wasserstoff- und Hydroxylionen als Keimungsreize. Ber. Deutsch. Bot. Gesell. **25**:108–122. 1907.
12. FISCHER, MARTIN, Edema; a study of the physiology and the pathology of water absorption by the living organism. New York. 1910.

13. FRIEDENTHAL, HANS, Methoden zur Bestimmung der Reaktion tierischer und pflanzlicher Flüssigkeiten und Gewebe. ABDERHALDEN'S Handbuch der Biochemischen Arbeitsmethoden. pp. 534–566. 1910.
14. GASSNER, GUSTAV, Über Keimungsbedingungen einiger südamerikanischer Gramineensamen. Ber. Deutsch. Bot. Gesell. **28**:350–364. 1910.
15. ———, Über Keimungsbedingungen einiger südamerikanischer Gramineensamen. Ber. Deutsch. Bot. Gesell. **28**:504–512. 1910.
16. ———, Vorläufige Mitteilung neuerer Ergebnisse meiner Keimungsuntersuchungen mit *Chloris ciliata*. Ber. Deutsch. Bot. Gesell. **29**:708–722. 1912.
17. GREEN, J. REYNOLDS, On the germination of the castor-oil plant. Proc. Roy. Soc. **48**:370–392. 1890.
18. ———, and JACKSON, H., Further observations on the germination of the castor-oil plant. Proc. Roy. Soc. **77**:69–85. 1905.
19. GUIGNARD, L., Sur la metamorphose des glucosides cyanhydriques pendant la germination. Compt. Rend. Sci. Paris **147**:1023. 1908.
20. HEINRICHER, E., Keimung von *Phacelia tanacetifolia* und das Licht. Bot. Zeit. **67**:45–66. 1909.
21. HEMPEL, J., Researches into the effect of etherization on plant metabolism. Kgl. Danske. Vidensk. Selsk. Skrift VII. **6**:215–277. 1911.
22. HOWARD, W. L., An experimental study of the rest period in plants. The winter rest; initial report on the treatment of dormant woody plants for forcing them into growth. Research Bull. 1. Agric. Exp. Sta. Univ. Missouri. 1910.
23. IRAKLIONOW, P. P., Über den Einfluss des Warmbads auf die Atmung und Keimung der ruhenden Pflanzen. Jahrb. Wiss. Bot. **51**:515–539. 1912.
24. IVANOW, SERGIUS, Über Oelsynthese unter Vermittlung der pflanzlichen Lipase. Ber. Deutsch. Bot. Gesell. **29**:595–602. 1911.
25. ———, Über die Verwandlung des Oels in der Pflanze. Jahrb. Wiss. Bot **50**:375–386. 1912.
26. ———, Über den Stoffwechsel beim Reifen ölhaltiger Samen mit besonderer Berücksichtigung der Ölbildungsprozesse. Beih. Bot. Centralbl. **28**: 159–191. 1912.
27. JESENKO, F., Einige neue Verfahren der Ruheperiode die Holzgewächse abzukurzen. Ber. Deutsch. Bot. Gessell. **29**:273–283. 1911.
28. ———, Über das Austreiben im Sommer entblätterter Bäume und Sträucher. Ber. Deutsch. Bot. Gesell. **30**:226–232. 1912.
29. JOST, L., Plant physiology. Eng. trans. 1907.
30. JOHANNSEN, W., Das Äther Verfahren beim Frühtreiben. 2. Aufl. Jena. 1906.
31. KIESSLING, L., Experiments on the germ-ripening of grain. Landw. Jahrb. Bayern. **6**:449–514. 1911.
32. KINZEL, W., Über die Keimung halbreifer und reifer Samen der Gattung *Cuscuta*. Landw. Vers. Stat. **54**:125–134. 1900.

33. KLEBS, G., Wilkürliche Entwicklungsänderungen bei Pflanzen. 1903.
34. ———, Über die Rhytmik in der Entwicklung der Pflanzen. Sitzr. Heidelb. Akad. Wissh. **23**: 1911.
35. LAKON, GEORG, I. Der Keimverzug bei den Koniferen- und hartschaligen Leguminosensamen. Naturwiss. Zeitschr. Forst- und Landw. **9**: 226–237. 1911.
36. ———, II. Zur Anatomie und Keimungsphysiologie der Eschensamen. *Ibid.* **9**:285–298. 1911.
37. ———, Die Beeinflussung der Winterruhe der Holzgewächse durch die Nährsalze. Zeitschr. Bot. **4**:561–582. 1912.
38. MANGHAM, S., On the detection of maltose in the tissue of certain Angiosperms. New Phyt. **10**:160–166. 1911.
39. MILLER, E. C., A physiological study of the germination of *Helianthus annuus*. Ann. Botany **24**:693–726. 1910.
40. MOLISCH, H., Über ein einfaches Verfahren Pflanzen zu treiben (Warmbadmethode). Sitz. Kais. Akad. Wiss. **117**:87–117. 1908.
41. ———, Über das Treiben von Pflanzen mittelst Radium. Sitz. Ber. Akad. Wien **121**:121–139. 1912.
42. MÜLLER-THURGAU, H., und SCHNEIDER-ORELLI, Beiträge zur Kenntniss der Lebensvorgänge in ruhender Pflanzen. 1. Über den Einfluss der Vorerwärmens und einiger anderer Faktoren. Flora **101**:309–372. 1910.
43. ———, Beiträge zur Kenntniss der Lebensvorgänge in ruhenden Pflanzenteilen. Flora **104**:386–446. 1912.
44. NOBBE, F., Handbuch der Samenkunde. 1876.
45. NOBBE und HANLEIN, Über die Resistenz von Samen gegen die äusseren Factoren der Keimung. Landw. Versuchs. Stat. **20**:63–96. 1877.
46. PAMMEL, L. H., and KING, CHARLOTTE M., Delayed germination. Proc. Iowa Acad. **17**:20–33. 1910.
47. PROMSY, G., De l'influence de l'acidité sur la germination. Compt. Rend. Acad. Sci. Paris **152**:450–452. 1911.
48. ———, De l'influence des acides organiques et du glucose sur la respiration des graines en voie de gonflement. Rev. Gén. Botanique **23**:313–318. 1912.
49. RAVENNA, C., and VECCHI, C., Hydrocyanic acid formation in germination of seeds. Atti R. Accad. Lincei V. **2**:491–495. 1911.
50. SCHMIDT, R. H., Über Aufnahme und Verarbeitung von fetten Oelenpflanzen. Flora **74**:300–370. 1891.
51. WALLERSTEIN, M., Die Veränderungen des Fettes während der Keimung und deren Bedeutung für die chemisch-physiologischen Vorgänge der Keimung. Abst. Chem. Centralbl. **1**:63. 1897.
52. WIESNER, J., Biologie der Pflanzen. II. Aufl. pp. 51 ff. 1902.
53. WEBER, F., Über die Abkurzung der Ruheperiode der Holzgewächse durch Verletzung der Knospen beziehungsweise Injektion derselbe mit Wasser. Anz. Kais. Akad. Wien **10**:182–183. 1911.